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MULTIPLE SPAWNING AND MOLT SYNCHRONY IN A FREE SPAWNING SHRIMP (*SICYONIA INGENTIS:* PENAEOIDEA)

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ABSTRACT

Field sampling was conducted to examine patterns of molt and reproductive activity in populations of the free spawning, penaeoid prawn, *Sicyonia ingentis;* some of these patterns were also verified by laboratory studies. Multiple spawning in the field was inferred from the high frequency of prominent oocyte features which are termed cortical specializations. In a prior laboratory study (Anderson *et al.,* 1984), the time from cortical specialization stage to spawning was determined to be less than one week.

Molt studies showed that females began a synchronous molt cycle in June which was not completed until late October or early November, after the spawning season was over. Males exhibited similar synchrony, but the period was shorter, and there was more variability. Data indicate that not only the absolute duration of the molt cycle varied with season, but the relative duration of molt cycle stages also varied. December and January were the only months of the year in which the relative frequency of molt cycle stages approximated literature values.

Potential multiple spawning throughout the summer, coupled with almost no molting in the summer, suggest that multiple spawning occurred without intervening molt or mating in field populations. This was substantiated by laboratory studies which directly demonstrated multiple spawning by these prawns within a single, prolonged molt cycle.

INTRODUCTION

The relationships between molt and reproduction in the Crustacea have been studied for decades (Adiyodi and Adiyodi, 1970). Both proximate and ultimate factors which may potentially determine molt and reproductive patterns have been considered. The proximate endocrine factors have received the most attention (Adiyodi and Adiyodi, 1970; Kleinholz and Keller, 1979, for reviews). However, the roles of ultimate ecological forces and environmental variables have also been examined (*e.g.,* Aiken, 1969; Cheung, 1969; Reaka, 1976; Steel, 1980; Webster, 1982; Nelson *et al.,* 1983; Quackenbush and Herrnkind, 1983).

Much research has been done on molt and reproduction in the Crustacea, but most of this work has been conducted on brooding species. Remarkably, relationships between molt and reproduction in free spawning crustaceans have not been well characterized. The most frequently studied free spawners are the prawns of the families Penaeidae and Sicyoniidae, although all members of the superfamily Penaeoidea (suborder Dendrobranchiata) exhibit this characteristic (for recent tax-

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Abbreviations: GSI, gonadosomatic index; CL, carapace length; CS, Cortical specializations.

onomic revisions see Bowman and Abele, 1982). In the Penaeoidea, sperm are transferred by deposition of either an internal or external spermatophore and fertilization occurs at spawning.

The few studies (Emmerson, 1980; Penn, 1980; Crocos and Kerr, 1983) which have addressed the interrelationships of molt and reproduction in Penaeoidea have left two interesting questions unanswered. First, is multiple spawning within a single molt cycle possible in field populations, and second, what is the relative duration of molt cycle stages in field populations? Emmerson (1980) provides the only evidence that multiple spawning of both eyestalk-ablated and intact prawns can occur within a single molt cycle. Emmerson (1980) examined spawning induction in the laboratory but did not include an analysis of multiple spawning with respect to molt activity in the local field population. Crocos and Kerr (1983) state that there is no evidence that multiple spawning can occur within a single molt cycle in the field.

Knowledge of the timing of oocyte development is used in this work to study multiple spawning in field populations of the ridgeback prawn (*Sicyonia ingentis:* Sicyoniidae), an abundant benthic organism in the Southern California Bight. Anderson *et al.* (1984) employed an ovarian biopsy technique in the laboratory to determine the relative timing of postvitellogenic ovarian stages for *S. ingentis.* They found that after vitellogenesis, approximately four days are required to pass through the most advanced oocyte stages; that is from the time when the cortical specializations (CS) are fully formed until spawning.

To our knowledge, no one has followed the pattern of molt cycles and reproductive stages through time in any field population for the Penaeoidea. Emmerson (1980), however, provided information on the coincidence of the various molt stages with given ovarian stages in laboratory populations of *Penaeus indicus,* and Crocos and Kerr (1983) provided similar information for field populations of *P. merguiensis.* This then, is the first study to examine the relative duration of molt cycle stages in the field for any of the Penaeoidea. Furthermore, these field observations on molt cycles and multiple spawning are verified in laboratory studies.

Materials and Methods

Animal collection and field sampling regime

Field observations on reproduction and molting were made monthly for two years (15 November 1979 to 13 November 1981) and on an irregular basis during the spring and summer of 1982. The monthly sampling was conducted at one fixed station in the Santa Barbara channel off of Santa Barbara, California (Fig. 1). Commercial shrimp semiballoon trawl gear was used. Nets were approximately 30 m long and 7 m wide at the mouth. The mesh at the cod end was approximately 11.5 cm on the diagonal. The fixed station was located (Fig. 1) in 145 m of water approximately 25 km offshore. Random trawls were also made on some of the same dates as trawls at the fixed station (Fig. 1).

Monthly samples included two groups of animals. First, approximately 50 live prawns were selected randomly from the trawl. Second, a group of approximately 100 animals was taken from the same tow each month and frozen. Both live and dead animals were selected in this second group to prevent underrepresentation of postmolt animals due to death in the trawl. In the first group (the live sample), body wet weight, ovary wet weight, carapace length, molt stage, and presence or absence of CS were determined. In the second group (the frozen sample), sex,

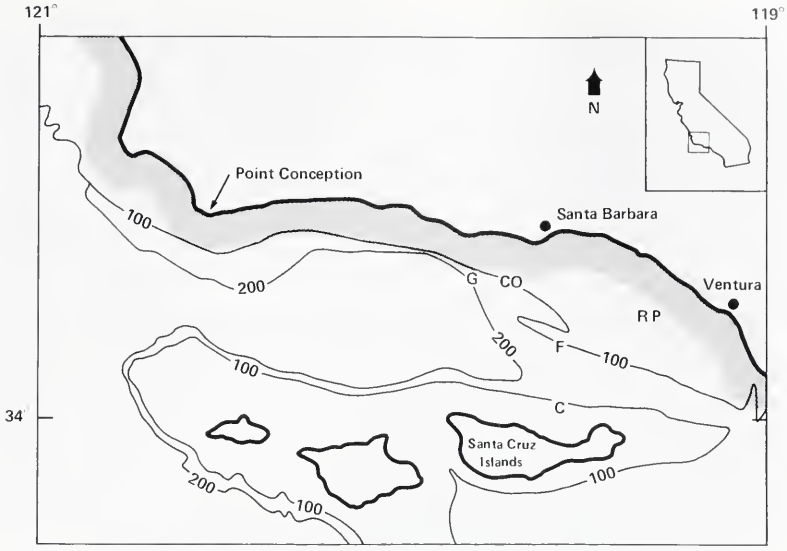


FIGURE 1. Study area in the Santa Barbara Channel. Insert shows the location of the Santa Barbara Channel along the coast of California. Shaded area denotes the three mile (4.8 km) limit. Depths in meters. F = fixed station (145 m), R = Rincon Pt. (60 m), P = Pitas Pt. (80 m), G = Gaviota area (220 m), CO = Coal Oil Pt. (150–250 m), C = China Cove (180 m).

carapace length, gross softness (indicating molt activity), and gross ovarian development were observed. Further details are given below.

Reproduction

The spawning season was delineated in two ways. First, gonadosomatic index (GSI) was determined for 20–30 live females monthly. GSI is defined as follows (Giese, 1958):

$$\frac{\text{ovarian wet weight}}{\text{body wet weight}} \times 100 = \text{GSI}$$

Animals were dried with paper towels until no water drained from the branchial cavity. Body and ovarian wet weights were recorded. Second, a gross estimate of reproductive activity was obtained by counting the number of ripe females in the frozen sample of approximately 100 animals. Shrimp with a green ovary extending from the base of the eyestalks to the base of the telson were recorded as ripe. The ovary is initially cream-colored. As oocyte development progresses, it gradually becomes green without any intermediate coloration. Therefore, animals scored as ripe are advanced vitellogenic or postvitellogenic. This corresponds to an "R" (ripe) stage ovary as described by King (1948). This gross estimate of ripeness was also determined on samples taken at random trawl sites.

The periods of spawning (1981 and 1982) were determined by microscopically observing ovarian fragments (from ovaries used for GSI determinations) for the presence of cortical specializations (CS); these are prominent oocyte features which form late in oocyte development (Duronslet *et al.*, 1975; Anderson *et al.*, 1984). Figure 2 shows a postvitellogenic oocyte which has not yet developed CS. Figure 3

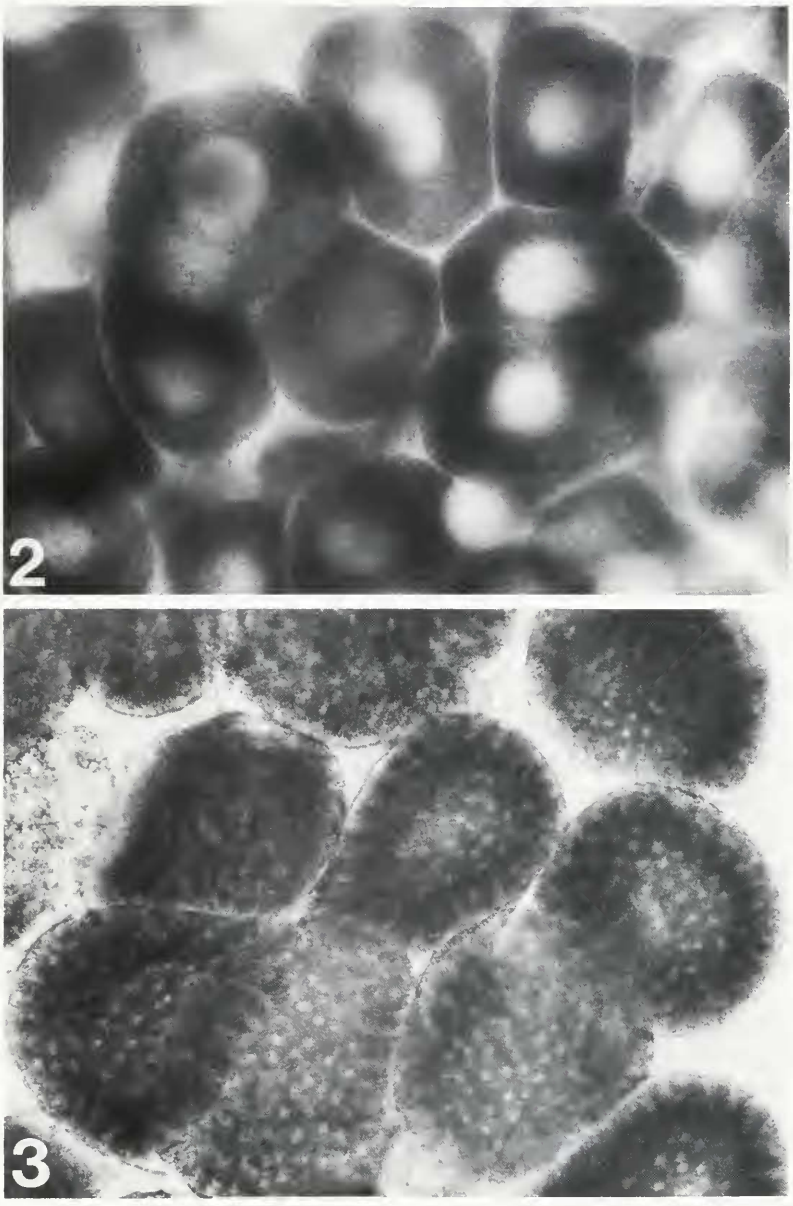


FIGURE 2. Postvitellogenic oocytes which have not yet developed cortical specializations (CS). 100×.

FIGURE 3. Cortical specialization (CS) phase oocytes. CS in these oocytes are the discrete light areas. 100×.

shows an oocyte with prominent CS which appear as discrete light areas. Presence of CS at the oocyte cortex indicates that spawning will frequently occur in less than one week (Anderson *et al.*, 1984). Consequently, CS serve as an index for spawning activity.

Molting

Molt activity was assessed in two ways. First, microscopic molt staging was performed on approximately 20 live females monthly (same animals used for GSI determinations) for two years and on approximately 20 live males monthly for one year. One antennal scale and one uropod were examined on each animal. Molt stage criteria are summarized in Table I. These criteria are modified from those of Drach (1939), Schafer (1968), Stevenson (1972), and Aiken (1973) specifically for application to *S. ingentis,* and the duration of the soft condition was determined by us in the laboratory. Molt stages were grouped into five categories (Figs. 4–7, Table I).

The second procedure for estimating molt activity, which was conducted at both the fixed and random stations (Fig. 1), was a determination of the percent of soft animals present in the sample of approximately 100 frozen animals. Soft animals are in stage A and part of stage B (Table I). This second means of estimating molt activity served as a comparison for determinations of the percent of animals in early postmolt taken from the live animal sample. These determinations were important because animals in early postmolt are very soft and are less likely to survive collection compared to their hardened cohorts. They are, therefore, less likely to be represented proportionately to their field abundance in a sample of live animals.

Morphological characteristics

Carapace length (CL) was measured from the posterior margin of the orbit to the posterior border of the carapace. Males were distinguished from females by the presence of the petasma (copulatory structure) in the former.

Multiple spawning and molt activity in laboratory populations

Two different laboratory approaches were taken to investigate the potential for multiple spawning occurrences without intervening molt or mating. One approach was to determine if sperm were retained in females after spawning and if so, whether these sperm represented an adequate amount to fertilize a clutch of eggs. To

TABLE I

Criteria for molt stage determination

Molt stage	Criteria
A-B	cones at base of setae not formed or just organizing carapace may be extremely soft (A) or only barely yield to pressure (B) soft condition lasts 24–48 h
C	cones at base of setae are being completed or are complete pigmented epidermis has not retracted from the cuticle
D_0	pigmented epidermis has retracted from the cuticle, retraction may be partial or epidermis may form straight line at base of setae or slightly below base of setae
D_1-D_2	epidermis slightly scalloped in appearance and/or new setae barely visible progressive stages of setagenesis occur
D_3-D_4	carapace loose or slightly loose and may be peeled off new setae are fully formed


5


7


4


6

accomplish this, four animals were killed immediately after spawning, and thelyca (sperm storage structure) were examined for the presence of retained sperm. Thelyca were dissected and homogenized lightly in 1 ml of cold crustacean Ringers (Pantin, 1934) with a Dounce homogenizer (B pestle). The homogenate was then vortexed, and sperm were counted using a hemacytometer. Additionally, an estimate of the number of eggs produced in a spawn was made to determine whether retained sperm represented an adequate quantity to fertilize a subsequent spawn. All of the eggs from six spawns were siphoned into formalin. Duplicate 5 ml aliquots of each spawn were counted and averaged.

The second approach used was to select animals just after spawning in the laboratory, to hold them in individual compartments (13 cm × 13 cm × 20 cm deep) of troughs supplied with running sea water (12–18°C, 33–34 ppt). These animals were monitored for egg development, spawning, and molt activity. The compartments were covered with clear plexiglass so that dim flourescent light reached the tanks 13–16 h per day. Animals were fed *Tubifex* and brine shrimp to excess. Deaths, spawns, and molts were recorded. Signs of ovarian development and resorption that were visible through the carapace were also recorded.

Embryos were examined approximately 18 h after spawning. Two samples of 30 embryos each were counted and averaged. The number of embryos which had passed through the 8-cell stage was recorded.

Statistical methods used to compare seasonal tendency to molt in the laboratory were the Bonferroni t procedure and the Scheffé's S test (Kirk, 1982).

Results

Reproduction

The spawning season was delineated by determining the frequency of ripe animals (n = approximately 100) in frozen samples and the mean gonadosomatic index (n = 20–30) on live animals taken in monthly trawls. Use of the gonadosomatic index to determine reproductive condition assumes that animals in the body size range studied have the same ratio of gonad size to body size, within a known limited range of variation in body size, at a given time of year, in a given locality (Gonor, 1972). To determine whether these data met such a condition, regression analyses were performed to determine whether GSI is dependent on body size. Log transformed GSI data were used because diagnostic plots of mean GSI *versus* variance showed that the variance increased with the mean. Log transformation effectively removed this dependence of variance on the mean. February and June of 1980 and 1981 were studied in order to compare periods of uniform high and uniform low ovarian bulk.

The relationship between GSI and body size was examined and in only one case out of four (June 1980), was a "*t*" test for the slope of the regression line of GSI

FIGURE 4. Molt stage A-B. Note the absence of setal cones. 50×.

FIGURE. 5. Molt stage C. Note the organization of the setal cones and the retraction of the epidermis. 50×.

FIGURE 6. Molt stage D_0. Note the retraction of the epidermis. 50×.

FIGURE 7. Molt stage D_1-D_2. Note the slight scalloping of the epidermis and presence of setal shafts. 50×.

versus CL significant ($P < 0.05$, Table II). Correlation coefficients, r, were not extremely low. These data suggest that any dependence of GSI on CL was weak or nonexistent. Regression of GSI on carapace length for all observations in the experiment (n = 613) was also performed. The slope was significantly different from zero ($P < 0.01$). However, the correlation coefficient was low (r = 0.105) and the number of observations was very large. Furthermore, the size range of animals in the area studied was narrow (22–45 mm CL).

The reproductive season for *S. ingentis* in the Santa Barbara Channel during 1980 and 1981 is outlined in Figure 8. Frequency of ripe animals closely paralleled the profile of log mean GSI. A period of low GSI was evident in the winter months from November until the end of April. Occasionally, animals with developed ovaries were seen at this time of the year, but they were not as well developed as during the peak season. GSI began to increase in late April, and this continued through May and into early June. This was the period when the first vitellogenesis of the season occurred. During late June, July, August, and September, high mean GSI was evident. The ovary accounted for 10–15% of the body weight. In these months, 75–95% of the population was ripe. A large drop in the number of ripe animals and ovarian bulk occurred in October and November, and the final spawning of the season occurred at this time. Similar results were obtained from samples taken at random stations.

Several lines of evidence raised questions as to whether these shrimp hold their eggs throughout the summer, have multiple spawns, or migrate into and out of spawning grounds. High mean GSI was maintained from late June through September, but the range in GSI values was very large. Moreover, 5–25% of the population was scored "not ripe" during these months. Examination of oocytes for CS showed a high incidence of this advanced stage throughout the summer, with maximum values in late July through early September (Table III). These data indicated a strong potential for multiple spawning during a single annual reproductive season.

TABLE II

Regression analyses examining the relationship between Log Gonadosomatic Index (GSI) and Carapace Length (CL) (for four individual months and for the overall study of two years of monthly observations)

Time period (n)	Regression equation Log GSI = a + b (CL)	r
Feb 1980 (n = 20)	a = 0.958 ± 0.766 b = −0.015 + 0.020	0.176
Feb 1981 (n = 30)	a = −0.083 ± 0.492 b = 0.027 ± 0.013	0.359
June 1980 (n = 30)	a = 0.056 ± 0.109 b = 0.006 ± 0.003	0.366*
June 1981 (n = 30)	a = 0.079 ± 0.3600 b = 0.020 ± 0.010	0.342
Overall (n = 615) 1980–1981	a = 0.149 ± 0.172 b = 0.013 ± 0.005	0.105**

* Signficant at $P < 0.05$.
** Significant at $P < 0.01$.

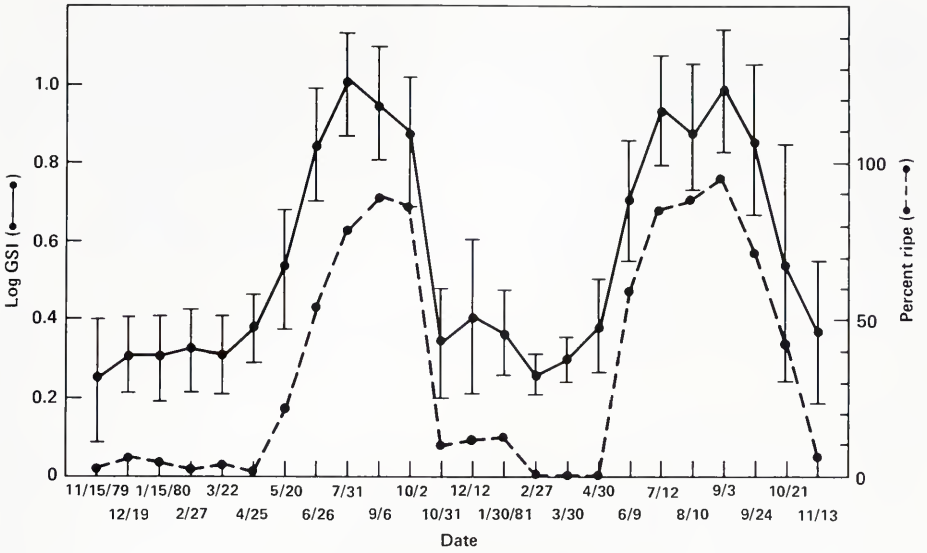


FIGURE 8. The reproductive season for *S. ingentis* in the Santa Barbara Channel (1980–81). Solid line is mean and standard deviation of log transformed monthly GSI values. Broken line represents mean percent ripe as judged by the presence of a green ovary in each month.

To determine whether frequency of occurrence of CS was uniform between size classes, the hypothesis that animals with CS differed in size from the mean size of the entire sample was tested using a "*t*" test. The mean carapace length of all animals in a given sample was tested against the mean carapace length of those with CS. In four out of six samples, the means were not significantly different (Table IV). Although the data remain inconclusive, there is apparently no strong association between incidence of CS and size, within the range tested.

TABLE III

Incidence of Cortical Specializations (CS)

1981*		1982*	
Date	% with CS	Date	% with CS
6/9	0	7/12	30
7/13	3	7/26	57
8/11	25	8/25	48
9/4	50		
9/25	25		
10/22	20		
11/14	0		

* Data for 1981 were collected at one fixed station and were taken from live animals. Data for 1982 were supplementary to the main study. Samples were taken from random stations, and ovarian fragments were formalin fixed. Results between the two years are not directly comparable.

TABLE IV

Size specificity of the frequency of Cortical Specializations (CS)

Date	Carapace length for the entire sample Means in mm (n)	Carapace length for animals with CS Means in mm (n)
9/4/81	37.0 (20)	36.6 (10)
9/25/81	34.7 (20)	36.0 (5)
10/22/81	35.0 (20)	33.2 (4)
7/9/82	36.7 (26)	40.3 (8)
7/26/82	35.5 (29)	37.7 (15)
8/25/82	35.6 (29)	35.8 (14)

* $P < 0.01$.

Molting

The two year profile of male and female molt activity based on the frequency of soft animals is given in Figure 9. For females, a high and broad peak of molt activity occurred both years between late February and early May. The exact frequencies of soft animals, however, varied between the two years. Between late October and early November of each year, another smaller peak in molt activity was observed; a slightly elevated frequency of soft animals was also observed in December. Throughout the summer, no soft females were observed. For males, no discernible peaks in molt activity were detected. However, soft animals were rarely (1980) or never (1981) seen during the summer. Data taken at random stations generally agreed with the data taken at the fixed stations.

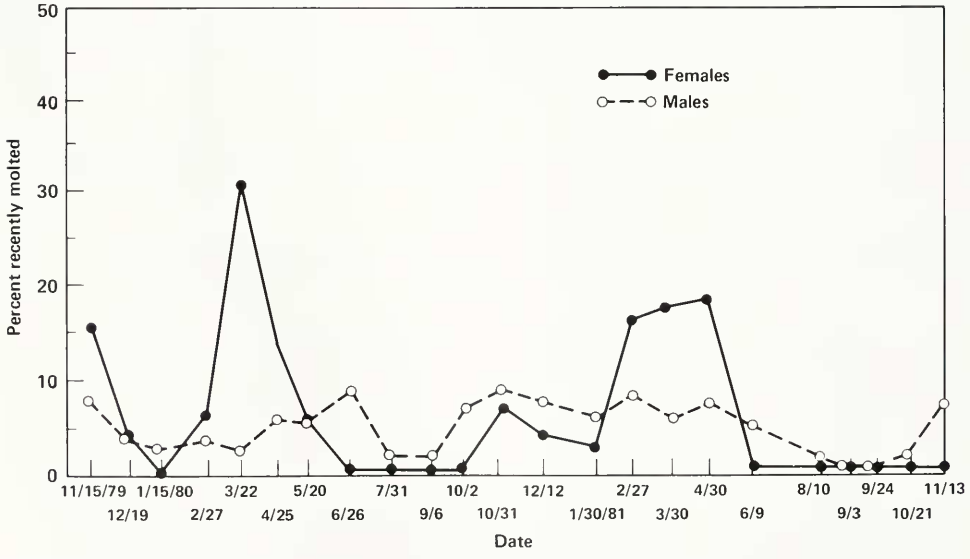


FIGURE 9. Molt activity (based on the frequency of soft animals) for *S. ingentis* in the Santa Barbara Channel. Solid and broken lines indicate data taken on females and males respectively at one fixed station.

Monthly determinations of molt stages were made microscopically for two years on females and for one year on males (Fig. 10). During November 1979, the period of the fall molt, 80% of the animals observed were in D_3-D_4. From December through January, 50–80% of the animals were in D_0 with lesser representation of other stages. The pattern for December and January roughly coincided with the expected relative duration of molt cycle stages as described in other shrimp species (Drach, 1944; Scheer, 1960). In March and April, no single molt stage predominated, and the frequency of the low probability stage, A-B, was relatively high. This indicates a very high frequency of molting, and it also indicates variability in length of the molt cycle. The high frequency of molting is corroborated by the data in Figure 9.

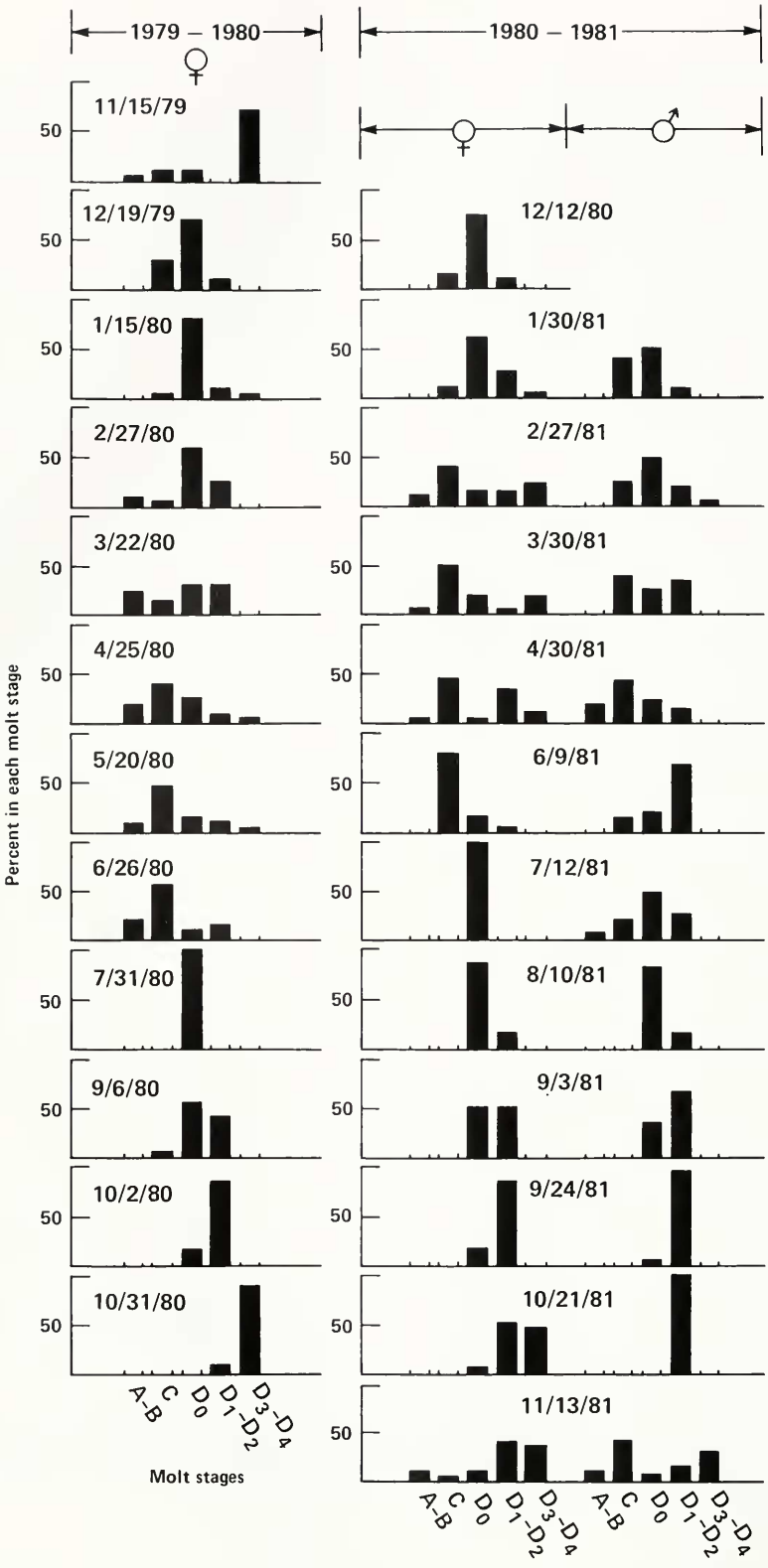
After the animals became vitellogenic in May, the range of observed molt cycle stages declined. In June, 50% were in stage C; by the end of July, 100% were in D_0. In September and October, animals were increasingly observed in later premolt stages. By late October (as with the previous November), nearly all animals were in D_3-D_4. These data indicate that a synchronous progression through a single prolonged molt cycle occurred. These data further indicate variability not only in length of the molt cycle but also in the relative duration of molt cycle stages. Data for females in 1980–1981 were nearly identical to 1979–1980 data already described, indicating that the observed patterns were reproducible.

Molt patterns for males from January through June (Fig. 9) indicated asynchronous molt activity. This is generally corroborated in the microscopic molt stage data; however, it is not known why a higher representation of molt stage D_0 or even C was not observed. In August, when the frequency of soft males declined to zero (Fig. 9), a synchronous progression through postmolt was initiated. This is similar to the data for females, except that the duration of the long premolt period was one month shorter.

For both males and females, microscopic molt staging indicated that the fall molt occurred between 21 October 1981 and 13 November 1981 (Fig. 10). This molt was never detected in an increased frequency of soft animals at the fixed station (Fig. 9). This indicates that the fall molt may have occurred very rapidly and could have been missed when only the frequency of soft animals was recorded.

Comparison of the two indices (Figs. 9 and 10) shows that Stage A-B animals were underrepresented in some of the live animal (microscopic staging) samples. However, this does not have much effect on the overall interpretation of the molt stage data. For the females during the period of anecdysis, there were zero molts so there is zero error. On 22 March 1980, 30% soft animals was recorded in the molt frequency sample, and 25% Stage A-B was reported in the live animal (microscopic staging) sample. Whereas in February, March, and April of 1981, 16–19% soft was reported and only 5–10% of the animals were in molt Stage A-B. Stage A-B was under-represented but the molt frequency (percent soft) data provide insight to this problem. During the months of December and January, when molt frequency was low and there was a spread of molt stages present, only the molt frequency (percent soft) sample with the high number of observations included the postmolt animals. In males, where synchrony was lower, there was approximately 5% underrepresentation of Stage A-B during the entire winter and spring.

Indication of a strong potential for multiple spawning throughout the summer, coupled with the observation of near-zero molt in the summer, indicated that multiple spawning occurred without intervening molt or mating. This possibility is further addressed in the next section.


1979 – 1980
1980 – 1981
♀
♀
♂
11/15/79
12/19/79
1/15/80
2/27/80
3/22/80
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5/20/80
6/26/80
7/31/80
9/6/80
10/2/80
10/31/80
12/12/80
1/30/81
2/27/81
3/30/81
4/30/81
6/9/81
7/12/81
8/10/81
9/3/81
9/24/81
10/21/81
11/13/81
50
Percent in each molt stage
A-B
C
D0
D1-D2
D3-D4
Molt stages

Multiple spawning potential and molt activity in laboratory populations

The purpose of the sperm enumeration work was to determine whether *S. ingentis* could potentially spawn multiple batches of fertile embryos without an intervening molt or mating. The number of sperm in four freshly spawned females ranged from 3×10^6 to 40×10^6 with a mean of 23×10^6. The number of embryos produced in 6 spawns ranged from 47,000 to 131,000 with a mean of 86,000. The size of animals also varied (25.4–35.7 mm CL). In any case, the lowest number of sperm present in a spawned female was more than an order of magnitude greater than the number of embryos in the largest spawn. These initial results provided a strong indication that multiple spawning without an intervening molt or mating was possible.

The second approach to the analysis of potential multiple spawning without an intervening molt involved laboratory observation of spawning and molt activity. The first group (6 August 1982), consisted of 14 females which were isolated after spawning in the laboratory. Fifty percent of these females spawned again (Table V), despite the fact that no special conditioning regimes were employed. The time between observed spawns was 19 ± 2 days (mean $\pm$ S.D.). No prawns molted before spawning, and the time to molt for the 11 animals which survived to molting was 52 ± 14 days (mean $\pm$ S.D.). The embryo quality data showed that these animals spawned viable ova which were successfully fertilized and developed well. Embryo quality values were comparable to those reported for recently field collected animals (Anderson *et al.*, 1984). For the 20 August and 22 September groups, multiple spawning was observed again; and in no case, did an animal molt before it spawned (Table V).

Field results indicated that prawns began to molt in the fall as the spawning season drew to a close (Figs. 9, 10). This result was corroborated in laboratory results (Table V). Animals collected in early August did not molt in the laboratory for nearly two months, whereas those collected in late September molted within one month. Moreover, females collected in September showed a decreased tendency to spawn. Most laboratory molting, then, occurred throughout the month of October after the spawning season was over. Similarly, peak molting in the field occurred in late October and early November when spawning was completed. These seasonal changes in time to molt and tendency to spawn were statistically analyzed.

When the hypothesis that mean time to molt was the same for the three groups was tested by one-way ANOVA (Table V), this hypothesis was rejected ($P < 0.0001$). Subsequently, pairs of means were compared in Bonferroni trials with the following results: 6 August *versus* 26 August, $P = 0.0143$; 6 August *versus* 22 September, $P = 0.0001$; 26 August *versus* 22 September, $P = 0.0014$. A Scheffé contrast was used to determine whether mean time to molt decreased with time ($\mu_1 > \mu_2 > \mu_3$, $P < 0.01$). These results demonstrate that mean time to molt decreased significantly as the spawning season came to an end.

When the hypothesis that the ratio of spawns was the same for the three groups was tested by a 2×3 contingency table (Table V), the null hypothesis could not be rejected ($0.05 < P < 0.10$). However, the percent of animals which exhibited repeated spawning was 50, 42, and 10 for the three groups. Although the result was not statistically significant at the $P < 0.05$ level, it is believed that this is primarily due to the biologically significant fact that few spawns occur late in the season, making

FIGURE 10. Microscopic molt stage patterns for *S. ingentis* in the Santa Barbara Channel. The frequency of molt stages is given for females (1979–1981) and males (1980–1981).

TABLE V

Incidence of multiple spawning without an intervening molt in laboratory populations of S. ingentis

	Collection date		
	8/6/82	8/26/82	9/22/82
Number observed[a]	14	12	10
Number that spawned[b]	7[c]	5[d]	1[e]
Time to spawn (days): Mean ± S.D.	19 ± 2	19 ± 8	14
Number that molted	11	9	7
Time to molt (days):[b] Mean ± S.D.	52 ± 13	39 ± 6	28 ± 5

[a] Prawns observed for spawn and molt activity all spawned once in the laboratory after field collection. They were then placed in the holding facilities used for this experiment.

[b] The hypothesis that the ratio of spawns was the same for the three groups was tested in a 2×3 contingency table ($\chi_{calc}^2 = 4.807$, $0.5 < P < 0.1$). The hypothesis that the time to molt was the same for the three groups was tested in one-way ANOVA ($P < 0.0001$). Subsequently, the hypothesis that $\mu_1 > \mu_2 > \mu_3$ was tested using the Scheffé's multiple comparison procedure ($P < 0.01$).

[c] Six of these prawns survived to molt. None molted before spawning.

[d] Three of these prawns survived to molt. None molted before spawning.

[e] This prawn survived to molt. It spawned before molting.

it difficult to detect statistically significant effects without a very high number of observations.

Molt stage and reproductive activity

Individual records on field collected and laboratory maintained animals revealed that vitellogenesis occurred in molt stages late B through D_1-D_2, and spawning occurred in stages C through D_1-D_2. As the animals progressed into D_3-D_4, ovaries were either spent or resorptive.

DISCUSSION

Spawning season

The spawning season of *Sicyonia ingentis* in the Santa Barbara Channel is from June through October with possible multiple spawning occurring throughout the summer (Fig. 8; Tables III, V). Although regression analyses examining the relationship between GSI and body size (Table II) were somewhat contradictory, we have no reservations about the use of GSI to delineate the reproductive season. This is based upon the observation that frequency of ripe animals gave the same results as did the GSI data (Fig. 8). Random station data were in agreement with data taken at the fixed station. Therefore, it seems valid to generalize the spawning season for the majority of the Santa Barbara Channel region.

The spawning period of *S. ingentis* is more highly seasonal than that of other grooved Penaeoidea on the Atlantic and Gulf Coasts of the United States. The spawning period is restricted to 4 months, and there is a very high incidence (75–95%) of ripe animals at any time within the season. Kennedy *et al.* (1977) studied rock shrimp populations (*S. brevirostris*) off the eastern coast of Florida. They found that spawning occurred year round with peaks in January through March. The maximum frequency of animals with developed ovary in any month was approximately 75%. The minimum was approximately 10% with 6–7 months of the year in the 20–30% range. Cobb *et al.* (1973) studied *S. brevirostris* on the western coast

of Florida. They also found year-round spawning. Peaks occurred in October through February. The maximum percent ripe (by adding their percent developing and percent ripe) was 65%. Numerous investigations on prawns of the genus *Penaeus* in Gulf and Atlantic coast waters also indicate year-round spawning for several species. This includes, but is not limited to, *Penaeus duorarum* (Cummings, 1961; Kennedy and Barber, 1981) and *Penaeus aztecus* (Renfro and Brusher, 1963).

Multiple spawning

The frequency of CS stage oocytes in ovaries of *S. ingentis* was used as an index of spawning activity in the field. Laboratory determinations (Anderson *et al.*, 1984) revealed that the time from the onset of CS phase to spawning ranges from 1 to 9 days with a mean of 4 days at ambient fluctuating temperatures of 12–18°C. It is not known how environmental parameters affect this rate. Nevertheless, in interpreting these data, it may be concluded that the majority of animals observed to be in CS phase will probably spawn within a week.

Assuming animals with CS will spawn within a week, then for every month in which at least 25% of the population was observed to be in CS phase, we can predict approximately one spawn per month for each animal. This criterion was met or exceeded in three months during 1981 (Table III). Attempts to examine any size specificity in the occurrence of CS were inconclusive (Table IV); therefore, we are not certain whether the frequency of repeated spawning is the same in all size classes.

Direct documentation of multiple spawning within a single molt cycle has been provided by Emmerson (1980) in laboratory studies on *P. indicus*. It was found that unablated, recently wild caught females could spawn up to three times without a molt. However, no field studies were conducted. Our laboratory studies were initiated halfway through the spawning season, and two spawns within one molt cycle were noted for 13 animals. It was also demonstrated that mean time to molt in the laboratory decreased as the spawning season in the field ended. The prolonged molt cycle which occurs in the summer made it possible for us to also infer multiple spawning within a single molt cycle in field populations.

An alternative explanation to the possibility of repeated spawning within a single prolonged molt cycle is that the selected sampling station is a spawning ground through which ripe prawns (possibly in a favored molt stage) move during the summer. This possibility cannot be entirely refuted; however, four lines of evidence argue against this possibility. First, the size distribution of both males and females at the sampling station did not change over two years (Anderson *et al.*, 1985). Second, although the sex ratio at the sampling site varied, no systematic pattern was detected (Anderson *et al.*, 1985). Third, data collected on molt and reproductive activity at random stations were in agreement with fixed station data. Fourth, laboratory data demonstrated the potential for repeated spawning within a single molt cycle (Table V), and animals collected in early August delayed their molt in the laboratory until October, when the spawning season in the field was drawing to a close (Table V).

Molting

Monthly analyses of two indices of molt activity for *S. ingentis* populations revealed patterns of molt activity which vary dramatically with changes in reproductive status of the prawns and with season (Figs. 9, 10). Molt frequency was highest in

the winter and spring months when ripe animals were rarely found. Molt frequency was especially high in the late spring, prior to the onset of the spawning season. Ovarian bulk rose rapidly in May. At this time, the majority of the females began to progress synchronously through a single molt cycle which was not completed until late October or early November, after the spawning season was over. Males exhibited similar synchrony, but the synchronous period was shorter and there was more variability. To our knowledge, this is the first documentation of such dramatic molt synchrony in a population of free spawners.

Although molt stage data (Fig. 10) indicate that a synchronous molt occurred in November after the reproductive season was over, this was not always reflected in a peak percentage of soft animals (Fig. 9). This peak was narrow, and the period of maximum molting can be missed. This means that if a synchronous molt occurs over a short time period, sampling only for soft animals may not be sufficient to routinely detect this transient peak. It is also possible that significant mortality occurs after the fall molt and this affects the height of the peak.

Other interesting findings of this study are, first, that during the reproductive anecdysis of this free spawner, all molt stages from D_0 through D_3-D_4 were prolonged, not just D_0. In contrast, investigators working on brooding species (Hiatt, 1948; Aiken, 1973) have noted that D_0 or C_4 are the stages which are prolonged when anecdysis occurs. Second, it was shown that not only the absolute duration but also the relative duration of molt cycle stages may vary with sex and season.

Drach (1944) and Scheer (1960) have used the proportion of field collected animals in a given molt stage as an indication of the relative duration of molt cycle stages in brooding prawns. We caution against the use of this method when collections are not numerous. Furthermore, it is important to note that different molt cycle stages imply behavioral changes which may affect results. The latter source of error could not be addressed in our study because of the great depth at which these prawns are found.

Interactions between reproduction and molting

Our observations on the co-occurrence of molt stages with selected reproductive conditions agree with those of Emmerson (1980) who found ripe *Penaeus indicus* in molt stages B-D_2. It was also noted that D_2-D_3 ovaries resorb to an undeveloped state. Aquacop (1975) also noted that molting animals were never ripe. The relationships described above only apply to free spawning species, and they should not be generalized to brooding crustaceans. For example, the brooding prawn *Macrobrachium nobilii* undergoes a molt immediately prior to extrusion (Pandian and Balasundaram, 1982).

Our data do not permit final conclusions on the ultimate ecological significance of the long period of molt synchrony in *S. ingentis* populations, but we would like to discuss five pertinent hypotheses. First, there is no cannibalism in this species, so the argument proposed by Reaka (1976) for stomatopods, that molt synchrony provides a temporal refuge from cannibalism, is unlikely in this case. Second, Klapow (1972) demonstrated semi-lunar rhythms of molting and reproduction of the isopod *Excirolana chiltoni*. He believed these rhythms optimized success of soft adults and hatchlings with respect to tidal exposure. The environment that *S. ingentis* inhabits poses no such problems. Third, it is possible that fall molt synchrony provides an opportunity for heightened mating success. However, this is not likely because the prawns will molt again, thus losing their sperm packet, before they become ripe the next summer. Fourth, molt synchrony may cause a predator

satiation effect. We feel this is an interesting hypothesis, possibly implying selection at the population level. However, this is a difficult hypothesis to test. Finally, it is possible that proximate factors, such as the physiological demands of egg production are of overwhelming importance in the determination of this molt synchrony phenomenon. This rationale for molt inhibition is not new (Adiyodi and Adiyodi, 1970), but it has not been effectively tested. The physiological demands of egg production are dictated by the reproductive strategy of the organism. In the case of *S. ingentis,* multiple spawning occurs throughout a single synchronous molt cycle. We suggest that the dramatic molt synchrony which occurred in this *S. ingentis* population during the spawning season may be related to their highly seasonal spawning activity and may not occur in populations of penaeoids with protracted spawning seasons and more rapid growth rates. It would be possible to test this hypothesis by determining whether both molt synchrony and multiple spawning occur only in penaeoids with restricted spawning seasons.

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